ZEBRA 2 - ZOOPLANKTON ENUMERATION AND BIOMASS ROUTINES FOR APIOS:

A SEMI-AUTOMATED SAMPLE PROCESSING SYSTEM FOR ZOOPLANKTON ECOLOGISTS

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INTRODUCTION

Aquatic ecologists and lake managers have an ongoing interest in freshwater zooplankton communities for both theoretical and practical reasons. Ecologists are interested in zooplankton because they are the primary pelagic herbivores in lakes, funnelling energy up the food web from phytoplankton to fish. In consequence, their morphology, taxonomy, growth, behaviour, demography and community interactions are inherently worthy of study. Lake managers are interested in zooplankton because they modify the clarity of lakes by removing algae, because small fish often rely on them as their principal food and because zooplankton have proven to be excellent indicators of both detrimental and beneficial changes in water quality (e.g., Keller and Yan 1991).

Zooplankton data are conventionally gathered by microscopic examination of preserved samples, a very time-consuming exercise. The task is especially daunting if, as is often the case, the animals must be identified, counted and measured. This difficulty has stimulated the development of computerized counting and measuring systems. Three types of systems exist - automated, non-optical systems (e.g., the Coulter counter), automated image-analysis systems (e.g., Rolke and Lenz 1984; Hoai 1991; Thomsen 1991; and Herman 1992), and semi-automated systems. When employing a semi-automated system, the user identifies the animal then positions the delimiters of the electronic callipers on either a video or a microscope image of sequential fractions of the sample. Roff and Hopcroft (1986) have reviewed the advantages and disadvantages of each class of counting system. The first two types have the advantage of speed, but if information is required at the species level, only the semi-automated systems will suffice.

The usefulness of semi-automated plankton counting and measuring systems is not currently limited by hardware. Several satisfactory variants exist (Sprules et al. 1981; Mills and Confer 1986; and Roff and Hopcroft 1986). The problem is software. There is no system which both efficiently manages the large amounts of data generated by counts of samples, and permits the customizing of counting protocols to satisfy the needs of students of

zooplankton taxonomy, growth, production and population or community ecology. Herein we describe a system called ZEBRA2, for Zooplankton Enumeration and Biomass Routines for APIOS (the Acid Precipitation in Ontario Study), which can satisfy both of these needs. All data are stored in DBase tables serviced by powerful file managers. Counts of samples can be customized by the user to allow the assignment of any number of meristic, mensural, categorical and text attributes to uniquely identified and measured individuals in the count.

To process a zooplankton sample in ZEBRA2, the user acts as the intelligent interface between two systems of hardware - a video image display system, and a computer-coupled measuring system. The video system consists of a high resolution video monitor connected to a video camera mounted on a microscope. We recommend using a camera developed for surveillance purposes because of their sensitivity and resolving power. The measuring system is one of two types of electronic callipers connected to a micro-computer driven by the ZEBRA2 software. In this report we provide a detailed description of the software.

ZEBRA2 was developed to satisfy the needs of the zooplankton component of the APIOS program of the Ontario Ministry of the Environment. This component was executed by Limnology Section staff at the Dorset Research Centre (DRC). Inevitably, the software bears the stamp of its development history. For example, its database structure complements that of Pawson and Yan (1993), the zooplankton database of the DRC. Secondly, the species reference table supplied with the program has been populated with lists of zooplankton species and length-weight data for Ontario fauna. Thirdly, a routine is included that permits the importing of count data from an earlier version of the software. Fourthly, a routine called "MOE disk" is included that permits the importing of descriptions of samples collected using the standard DRC sampling protocol described by Girard and Reid (1990). Fifthly, standard sampling protocols used by the DRC are built into the sample editor. Sixthly, while any groups of zooplankton can be counted, the focus is on Crustacea. Lastly, on-line help screens often provide example values of codes used by the DRC. These features should not limit the general use of the program, but they do mark it with a DRC stamp.

In the following pages, we describe the installation and use of ZEBRA2. Those wishing advice on the hardware components should contact their regular microscope suppliers for advice. The authors are also quite willing to supply advice. We do not describe how plankton samples should be counted. We assume that the system will be used only by knowledgable zooplankton ecologists who do not need technical guidance. For example, we assume that users will understand the danger of assuming that the length-weight relationships supplied with the SPECIES table are applicable to their study populations. With this gentle warning, we wish you happy zooplankton counting.

INSTALLATION

Hardware Requirements

The program will only run on IBM compatible machines. It will run on an XT (8088 CPU), but we recommend using at least an AT (80286 CPU) for comfortable operating speed. The speed of the program also depends greatly on the speed of your hard disk. 512K of RAM memory are required (after DOS and any memory resident programs are loaded); however, the program can make use of any extended or expanded memory in your system to speed up its operation.

At the moment, the system can employ three types of electronic callipers - manual, scissors and vernier. For manual callipers, animal sizes are simply keyed in when requested. In this case, ZEBRA2 simply functions as a sophisticated data logger. The scissors callipers are those described by Sprules et al. (1981). The callipers and associated A/D card can be ordered through the electronics shop at the Erindale Campus of the University of Toronto in Mississauga. Contact Dr. Gary Sprules (Zoology Department, University of Toronto, Erindale Campus) for cost information. The vernier callipers are Fowler Ultracal II callipers made by Fowler Tools.

Software Requirements

Any version of DOS after 3.0 will work but we recommend Version 3.3 or later. If you are using Version 4.0x, it is well worth your while to upgrade to Version 5.0 since it requires less memory, is relatively bug free and has several enhancements (in fact, we prefer Version 3.3 over 4.01!).

Program Installation

Installation of the program consists of copying the distribution files onto your hard disk and updating your CONFIG.SYS and AUTOEXEC.BAT files. If you are not comfortable with creating directories or do not know how to the edit these files, it would be a good idea to either spend some time reading your DOS manual or contact DataBase MicroComputing at 613-531-9870 and they can talk you through the installation over the phone.

Using this Manual

We have tried to be consistent in our documentation regarding the use of the keyboard. Always type the character or press the key indicated inside the angle brackets, < >. **Do not type the brackets themselves!**

Copying the Files

The distribution files must reside in a directory called ZEBRA2. It is not necessary that this be directly below the root directory, although this is the simplest approach. In the instructions that follow, the **C:** represents the appropriate drive letter for your hard disk and the **A:** represents the drive letter of the floppy drive containing the distribution disk.

At the **C:\>** prompt, type:

<**MD ZEBRA2**> then press the <**Enter**> key

Upper and lower case are not distinguished, so <md Zebra2> is equivalent to <MD ZEBRA2> in this documentation.

Now change to the new directory: type

<CD ZEBRA2> then press **<Enter>**

Next, copy the distribution files: type

<copy A:*.*> then press **<Enter>**

CONFIG.SYS FILE:

The following statement should appear in your CONFIG.SYS file:

FILES=35 (A number greater than 35 will do)

AUTOEXEC.BAT FILE:

The following statement should appear in your AUTOEXEC.BAT file:

SET CLIPPER=F35

Please note that there should not be any spaces on either side of the equals sign.

Starting the Program

If it was necessary to change either of the above files on your system, it should be re-booted before continuing. After re-booting, change to the ZEBRA2 directory, type **<ZEBRA2>** and press **<ENTER>**. This should start the program. The first time it is run, a message will appear at the bottom of the screen indicating that new indices are being built. This should only take a few seconds; then the Main Menu will appear.

GETTING STARTED

Using the Keyboard

We've already indicated that you should always type the keys within the angle brackets < > and treat upper and lower case as equivalent. Here are a few additional comments on the use of the keyboard.

Computer keyboards have several keys not found on a normal typewriter. Two of these, CTRL and ALT are like extended SHIFT keys and are usually used in conjunction with another key. This is represented in this manual by e.g., **<Ctrl><G>** which indicates that you should hold down the **<Ctrl>** key while pressing the **<G>** key. Other keys you will frequently use are the INSERT, PAGE UP, PAGE DOWN, END, DELETE and especially the ESCAPE key. For more comments on keyboard use, see Appendix 2.

Not Using this Manual

Based on a good deal of experience, we have come to the conclusion that many people do not like to read manuals. The expression "as a last resort, read the manual" seems to accurately reflect the approach of most users to a new program. As a result, we have written ZEBRA2 in a manner that makes this chore unnecessary, for the most part. By learning the use of 3 keys and skimming the next few pages, you can probably find your way through ZEBRA2 without much additional help from this manual.

<F1> will provide HELP on the current action to be performed.

<F10> will allow you to LOOK UP possible pre-defined entries related to the current action.

<Esc> will allow you to back out of virtually anything.

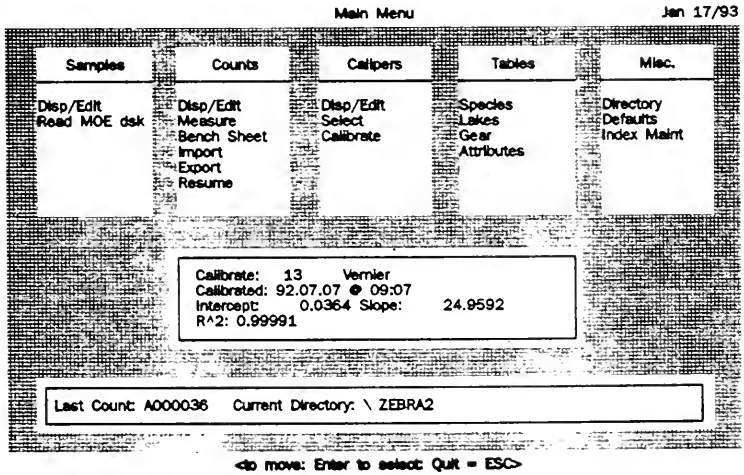
Selecting from Menus

Many ZEBRA2 functions are performed by selecting from "Light Bar Menus" in which several options are presented, one of which will be highlighted. For example, Figure 1 illustrates the appearance of the Main Menu when we logged into the program on January 17, 1993. You can simply move the highlight to the desired selection using the cursor keys, then press **<Enter>** to activate the selection.

Menu Structure

Unlike the majority of current programs, we have not implemented a Lotus (or CUA) style menu system. The advantage of our approach is that your choices are all evident from the initial screen. We find that this works better for new users who do not have to guess what options are available under all the "pull-downs", yet does not hamper experienced users because the system is very efficient to use.

Figure 1 - Illustration of Main Menu of ZEBRA2



Navigation

Menu items are selected by highlighting the desired element and pressing **<ENTER>**. Horizontal movement is accomplished by using the left and right arrow keys. The highlight on the Menu Titles will change to indicate which of the sub-menus is active. The menus "wrap" so that continuing to press the cursor key at either the extreme left or right of the screen will return you to the opposite side. Vertical movement within a sub-menu is accomplished either by using the up and down arrow keys or typing the 1st letter of the desired item.

Other

There is some additional information on the Main menu. The box immediately below the menu panels indicates the set of callipers selected (Vernier callipers #13 in this case) and the results of their last calibration. The box at the bottom indicates the project sub-directory and the identification code of the last counted sample, A000036 in this case.

OVERVIEW OF FEATURES

ZEBRA2 was designed to do the following eight major tasks:

1) produce and maintain four reference files: the first for all of the species you might encounter, the second for all of your study lakes, the third for all of your sampling gear, and the last for all individual attributes (e.g., gender, clutch size, the length of a specified body part, developmental stage) you might wish to employ during a count,

2) define and calibrate three types of electronic calipers used to measure animals during a count,

3) produce and maintain project sub-directories,

4) produce and maintain an electronic archive of your zooplankton samples, and counts of those samples within each project directory,

5) orchestrate the counting of samples, involving the identification and measurement of any number of animals, and the assignment of values of any other user-defined attribute to uniquely identified individuals,

6) produce and maintain a relational database of all counting sessions,

7) produce printed summaries of counts, and

8) export selected data to disk, for retrieval by other software packages.

The next four pages provide a summary of these features. The subsequent main body of this documentation explains how these tasks are executed in great detail.

Summary of Selections

What follows is a brief discussion of the purpose and use of each of the possible Main Menu selections (see Figure 1). More detailed information can be found later in the manual under the appropriate topic.

Samples Panel
Disp/Edit

Use this editor to view, delete, or edit information on the zooplankton samples themselves (e.g., sample date, crew chief, etc.) or to add new samples to the system.

Read MOE Disk

Use this feature to import sample collection data on a batch of samples from a diskette. This routine is only useful if the samples have been described in some system other than ZEBRA2 but are going to be counted by ZEBRA2. A subsidiary program called ZB_CKMOE supplied with ZEBRA2 allows the user to check the data prior to importing it to ensure the data are imported correctly.

Counts Panel

Disp/Edit

Use this editor to view, delete, or edit information on specific counts of samples or to describe additional counts of samples you will perform. Note that ZEBRA2 distinguishes a "sample", i.e., a bottle with preserved animals, from a "count" of a sample. One sample can be counted more than once, and with differing counting protocols.

Measure

This accesses the routines you will use to count the samples. Most of your time will be spent in this section of the program.

Bench Sheet

This is the routine that prints a summary of a completed count.

Import

This routine imports count data from a previous version of the program - ZEBRA1. Those that have not used this older version will never need this feature.

Export

This routines allows you to export count data from the system to a floppy disk either for archival purposes or to send results to another person. An option is provided to purge the exported data from your system after exporting them.

Resume

This routine allows you to return to the measurement screen of the last counting session with a single key stroke.

Callipers Panel
Disp/Edit

This editor permits the viewing, editing and description of existing or new electronic callipers.

Select

This routine allows you to select callipers for use.

Calibrate

This calibrates the callipers using a video image of a stage micrometer.

Tables Panel

The Tables routines facilitate the viewing, listing and modification (insertion, deletion, alteration of contents) of four important systems tables - one each for Species, Lakes, Gear and Attributes.

Species

This is the editor for the file describing all possible zooplankton species that could be encountered.

Lakes

This is the editor for the file listing all of the lakes sampled.

Gear

This is the editor for the file describing all zooplankton collection gear employed.

Attributes

This is the editor for the file describing all attributes of individual zooplankton that might be recorded during a count. Body length is not such an attribute. All animals counted are measured in ZEBRA2.

Misc. Panel
Directory

This routine allows you to select a project directory or create a new directory for a project. A project simply represents a block of related samples.

Defaults

Defaults for the most commonly employed callipers, microscope magnification factor, initials of enumerator, count protocol, count hardware, count type and group of organisms targeted

in the count are set using these routines. Configuration information, e.g., preferred Date Format and Printer, is also entered here.

Index Maintenance

It may be occasionally necessary to rebuild indices for the files, for example, if there is a power failure. Using this routine you can quickly rebuild the indices on the files.

DETAILED DESCRIPTION OF FEATURES

There are several steps involved in the customizing of ZEBRA2 to your needs and the subsequent counting of zooplankton samples, namely:

1) Modification of the supporting Species, Lake, Gear and Attribute tables.
2) Selection of system defaults.
3) Selection and calibration of the callipers.
4) logging samples into the system.
5) Logging counts into the system, performing the count, printing and/or exporting the results.

We will go through each of these operations in detail, titling each section with the associated panel header from the Main Menu (Figure 1).

1. TABLES

There are four tables, one each for zooplankton species, lakes sampled, sampling gear and individual attributes. To count a sample you must first ensure that the lake sampled and sampling gear have been logged into the Lake and Gear table. All species you encounter must be logged into the Species table, and all individual attributes you might wish to record must be logged into the Attribute table.

The command menu at the bottom of the editor for each of these tables permits you to add a new entry (**<INSERT>**), delete one (**<DELETE>**), edit an entry (**<ENTER>**), list selected entries (**<L>IST**), (**<I>MPORT**) entries or escape back to main menu (**<ESCAPE>**). Entries selected can be listed either to a printer or to an ASCII file. If you have make a modification to a table, each editor also asks you to confirm the modification by giving you three choices, i.e., **<R>e-enter**, **<A>ccept**, or **<C>ancel**. Only if you press **<A>** for Accept, or move the cursor to "A" and press **<ENTER>** will the table be modified. Appendix 1 provides complete documentation of all of the system and data tables for ZEBRA2.

Species

This table records a 3 digit code for each species, an abbreviated name for the species that will be used on the bench sheet and in the measurement window, the full latin binomial of the species, the parameters of a power function ($wt = aL^b$) relating body length (L in mm) to dry weight (wt in μg), and minimum and maximum lengths that are used as rough checks on individual measurements during a count. Girard and Reid (1990) record the length-weight regressions used by the DRC. When you press **<S>** from the tables panel on the main menu, Figure 2 will appear. If you then wish to edit an entry, for species 101 for example, (Figure 3), press **<Enter>**. In the power function, "a" is the weight multiplier, and "b", the weight exponent.

Figure 2 - Illustration of the Species Records Screen

Zebra II — Species Records — Jan 27/93

Species Code	Abbreviation	Full Name
101	Ac curv	Acantholeberis curvirostris
102	Ac harp	Acroperus harpae
103	Al affin	Alona affinis
104	Al cost	Alona costata
105	Al gutt	Alona guttata
106	Al inter	Alona intermedia
107	Al quad	Alona quadrangularis
108	Al rect	Alona rectangula
109	Alona sp	Alona sp
110	Bos long	Bosmina longirostris
111	Ce. lacus	Ceriodaphnia lacustris
112	Ce. megal	Ceriodaphnia megalops
113	Ce. pulch	Ceriodaphnia pulchella
114	Ce. retic	Ceriodaphnia reticulata
115	Cerio sp	Ceriodaphnia sp.
116	Ch bicor	Chydorus bicornutus
117	Ch piger	Chydorus piger

<INS>=Add <DEL>=Delete <ENTER>=Edit <L>ist <I>mport <ESC>=Exit

Figure 3 - Illustration of Edit Species Pop-up Screen

Edit Species

Species Code : 101
Species Abbrev.: Ac curv
Species Name : Acantholeberis curvirostris
Minimum Length : 0.200 Maximum Length : 1.500
Weight Mult. : 14.0800Weight Expon. : 1.9800

The DRC reserves the 100 series of species codes for Cladocera, the 200 series for Calanoida, the 300 series for Cyclopoida, the 400 and 500 series for Rotifera, and the 600 series for macrozooplankton such as *Chaoborus* and *Mysis*. The species codes employed by the DRC are listed in Pawson and Yan (1993).

Naturally, you can design whatever species codes you like, with the constraint that they must be 3 characters in length. However, if you wish to use the Rotifer Ranking routine, you must reserve the 400 and 500 series for Rotifera.

Lakes

This table records the full name and a four digit identity code of each lake sampled. Lake names need not be unique, but lake identity codes must be. The lakes are listed alphabetically by identity code, not lake name.

Gear

This table describes attributes of the gear employed to collect the samples. Press **<G>** from the tables panel to access the Gear Records screen (Figure 4). After assigning a unique gear code, the user must first identify the gear type. There are currently three permitted types of gear: C/B, S/P, and OTH.

The C/B gear (for modified Clarke-Bumpus), is the plankton net routinely used by the DRC, and described by Yan et al. (1992). If this gear type is indicated, ZEBRA2 assumes that the sample is a composite of the contents of a number of metered, vertical hauls from differing depths. During sample description, ZEBRA2 will prompt you to enter the haul lengths, the haul counts, and the count of a calibration haul assumed to have a length equal to the longest haul. From these inputs, ZEBRA2 will calculate the sample volume and filtration efficiency.

Figure 4 - Illustration of Gear Records Screen

Zebra II — Gear Records — Jan 27/93

Code	Type	Description	Mesh Size	Diam	Area	Volume
01	C/B	C/B Metered Net	76	0.1221	0.0117	0.0000
02	C/B	C/B Metered Net	76	0.1246	0.0122	0.0000
03	C/B	C/B Metered Net	76	0.1246	0.0122	0.0000
04	C/B	C/B Metered Net	76	0.1246	0.0122	0.0000
05	C/B	C/B Metered Net	76	0.1246	0.0122	0.0000
06	C/B	C/B Metered Net	76	0.1246	0.0122	0.0000
07	S/P	S/P Trap	80	0.0000	0.0000	0.0338
08	S/P	S/P Trap	76	0.0000	0.0000	0.0300
09	S/P	S/P Trap	30	0.0000	0.0000	0.0345
10	S/P	S/P Trap	35	0.0000	0.0000	0.0380
11	OTH	Conical Tow Net	250	0.6990	0.3837	0.0000
12	OTH	Conical Tow Net	80	0.4890	0.1878	0.0000
13	OTH	Closing Conical Tow	150	0.4500	0.0000	0.0000
14	OTH	Conical Tow Net	150	0.6990	0.3837	0.0000
15	OTH	Conical Tow Net	80	0.2930	0.0674	0.0000
16	C/B	Clarke-Bumpus	76	0.1246	0.0122	0.0000
17	OTH	Closing Reduc Conical Tow	80	0.5000	0.1963	0.0000

<INS>=Add <DEL>=Delete <ENTER>=Edit <L>ist <ESC>=Exit

If you select gear type C/B, you should enter the mesh size of the net, its mouth diameter and area into the gear table (see Figure 5). Obviously, you should not enter a volume or an efficiency in this case.

Figure 5 - Illustration of an Add Gear pop-up

```
        Add Gear Code
Gear Id     : 01
Gear Type   : C/B
Description: C/B Metered Net
Mesh(um)    :   76
Diameter(m): 0.1221
Area(m^2)   : 0.0117
Volume(m^3): 0.0000
```

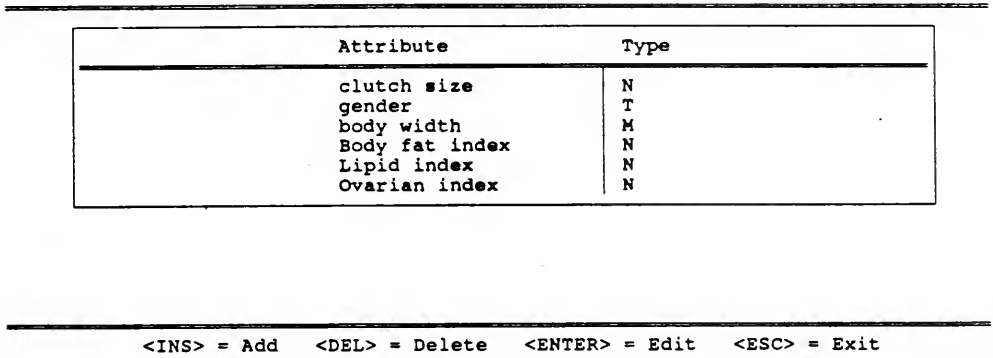
The S/P gear refers to a Schindler/Patalas or to any other type of box or bottle sampler, assumed to collect water from a discrete depth with a filtration efficiency of 100%. You should enter the trap volume and aperture size of the filtering mesh, but leave the mouth diameter and area blank. During sample entry, ZEBRA2 will prompt for the discrete depth from which the sample was taken. It will also prompt for the number of hauls that were taken from that depth, then calculate the sample volume from the trap volume and number of hauls.

The OTH type refers to any net other than the C/B used in the DRC manner. You should enter the net's mesh aperture size, its diameter and mouth area, and describe the net in detail. You should not enter a value for the sample volume. During sample entry ZEBRA2 will prompt for a sample volume and haul length. The program makes no assumptions about filtration efficiency for this gear, leaving it null. If you know the efficiency of the gear, use it to calculate the sample volume, i.e., enter the actual volume sampled, not the theoretical volume at 100% efficiency.

Attributes

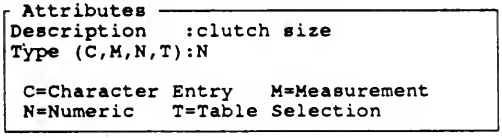
This routine (Figure 6) records the codes, types, and descriptions of individual zooplankter attributes other than body length. When you insert a new attribute, ZEBRA2 automatically assigns a unique identification code to this attribute.

Figure 6 - Illustration of the Attribute Editor Screen



You are then prompted (via a pop-up, Figure 7) to enter a 20 character description of the attribute. You are also prompted to identify the attribute type.

Figure 7 - Illustration of Attribute Editor Pop-up Screen



The possible types are:

[N]UMBER attributes - Numeric (counted not measured) parameters, such as numbers of eggs in a clutch or numbers of spines on an antenna. Numbered attributes are keyed in from the keyboard during a count.

[M]EASURED attributes - lengths of animal features other than the total animal length which you will record for every animal. An example might be a mucron length, head capsule length or egg diameter. Measured attributes are entered using the default callipers.

[T]ABLED attributes are those with a pre-set fixed number of possible values, gender for example. You will be prompted to supply all possible values for the attribute when you define an attribute type as tabular.

[C]HARACTER attributes are text strings attached to particular animals. You might see a gravid *Daphnia* with one of 6 eggs being degenerate. While clutch size, a numeric attribute, might be set to 5, for birth rate estimates, you might wish to add the comment "1 more rotting egg" to an individual animal comment, which would be a CHARACTER attribute.

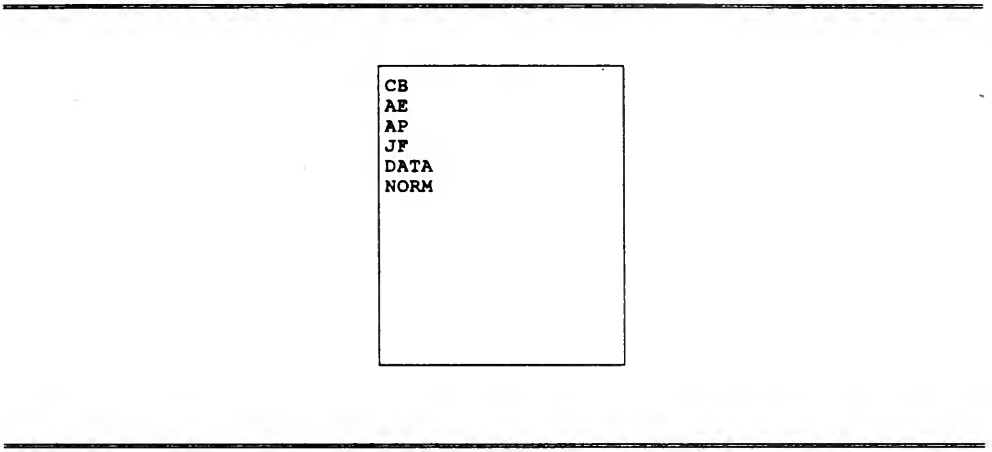
2. MISCELLANEOUS

After you have customized the reference tables to your initial needs, you should set some system defaults. Naturally, you can modify the reference tables or these defaults at any time. The Miscellaneous panel on the Main Menu (Figure 1) has three features: Directory, Defaults, and Index Maintenance.

Directory

This routine permits the user to select a data directory for the project from previously created directories, or to create a new data directory (Figure 8). You must select a data directory before logging in samples or initiating counts. When you create a data directory, ZEBRA2 creates a set of files (see Appendix 1) in the directory to record descriptions of the sample, counts of the sample, and the details of your counting sessions. We recommend you create directories only for such things as separate clients, major distinct blocks of samples, or major new classes of work.

Figure 8 - Illustration of the Project Directory Selection Screen

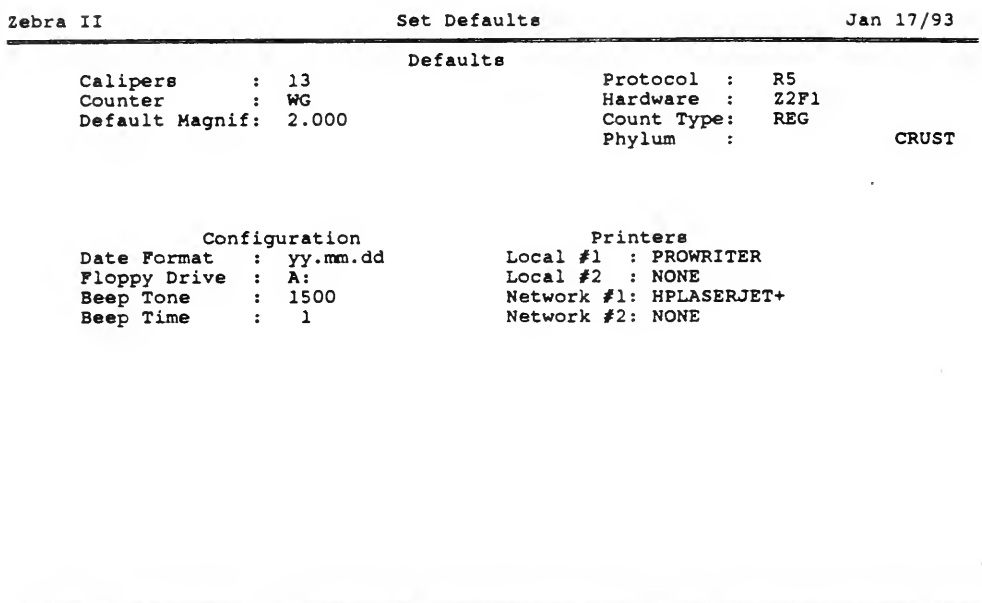


When you enter this routine, all current sub-directories of the ZEBRA2 directory are displayed. If there are more than will fit in the box, the list can be scrolled with the PGUP, PGDN, or the UP and DOWN cursor keys. To select a directory simply highlight the desired entry and press **<Enter>**. To create a new one, press **<Ins>** and provide a name for the directory.

Defaults

This routine allows the user to set numerous system and count default values (see Figure 9). The leftmost column of the upper portion of the screen permits the identification of the default callipers, taxonomist code and microscope magnification factor. Assign the default calliper code number into CALLIPERS. See the callipers features of the main menu to add new callipers to the system. Enter the initials of the person counting the samples into COUNTER. Next enter the default zoom ring setting or its analogue (see the discussion on magnification factor) into DEFAULT MAGNIFICATION.

Figure 9 - Illustration of the Set Defaults Screen



The right hand column of the upper portion of the screen permits the setting of defaults for the count protocol, count hardware, count type, and targeted groups of taxa in the count. Use the count PROTOCOL to code your default count strategy. Use the count HARDWARE code to identify all combinations of hardware used in the count. Use COUNT TYPE to identify the default class of count, for example, REG for regular, or QA for quality assurance. Then set Count PHYLUM to set a default code for the group of organisms targeted in particular counts.

The lower left hand portion of the screen permits the setting of a default drive, beep tone, and beep time. The default FLOPPY DRIVE will be the one normally used for importing and exporting of data. The BEEP TONE is the frequency used by the speaker. If you set

the default value lower than 250 or higher than 12,000 you will not be able to hear any of the warning beeps generated by the program. These warning beeps are very important! For example, ZEBRA2 will beep if an animal's measured length is not between the minimum and maximum allowable lengths logged into the species table for that taxon. This probably means your magnification factor is wrong. The beep tone is sounded for the number of seconds entered in BEEP TIME. Setting it to 0 will effectively turn the speaker off. We strongly recommend you do not do this!

This portion of the default screen also permits you to select a default format for entering dates. Options are:

British	dd/mm/yy
American	mm/dd/yy
ANSII	yy/mm/dd

We recommend you pick one and never change it.

You can also set a password using the default screen. In this version of ZEBRA2, the setting of privileges is fairly primitive. If you set a password, no one but you will be able to log into the program.

Index Maintenance

If you have a power failure during a session you may have to re-index your tables. You can do this using INDEX MAIN on the Miscellaneous panel of the Main Menu. When you begin this routine, Figure 10 will appear. Press **<F5>** to tag the files, then **<F7>** to re-index them and return to the Main Menu.

Figure 10 - Illustration of Index Maintenance Screen

3. CALLIPERS

This is the routine that permits the selection and calibration of the callipers. It has three features: Display/Edit, Select and Calibrate.

Display/Edit

In this section you can identify the codes and types of callipers you will use. At the moment there are only three possible types of callipers: manual, scissors and vernier. Mouse-driven systems will be added in the future. If manual callipers are selected, organisms lengths are simply entered into ZEBRA2 via the numeric keypad. However, the regression equation must still be generated using the Calibrate feature (below). The scissors callipers are those described by Sprules et al. (1981). They can be ordered through Dr. Gary Sprules of the Zoology Department at the Erindale Campus of the University of Toronto in Mississauga. The vernier callipers are Fowler Ultracal II.

Select

Use this feature to select one of the defined callipers. This selection will appear in the lower central panel of the Main Menu (Figure 1).

Calibrate

You calibrate the callipers by generating a linear regression equation relating actual sizes to sizes apparent on the video monitor. The actual sizes are preset in the system. They are 0.1 mm increments on a 2.0 mm stage micrometer. You will be prompted for 20 entries, i.e., for 0.1, 0.2, 0.3,....,2.0 mm (see Figure 11). Apparent sizes are taken from the display of the stage micrometer on the video monitor at a user-specified magnification factor.

Figure 11 - Illustration of Calliper Calibration Screen

Zebra II Calibration Jan 27/93

Operator : GGA
Zoom Ring Setting: 2.000

Enter caliper readings associated with stage micrometer readings of 0.1 mm to 2.0 mm in increments of 0.1 mm.

Actual Length in mm	Caliper Reading
0.1	0.00000
0.2	0.00000
0.3	0.00000
0.4	0.00000
0.5	0.00000
0.6	0.00000
0.7	0.00000
0.8	0.00000
0.9	0.00000
1.0	0.00000
1.1	0.00000
1.2	0.00000

Enter measurements, press <Ctrl><End> when done

If you are unhappy with any particular measurement, simply move the highlight to that micrometer setting, adjust the callipers, and re-enter the reading. Don't skip any entries. After you have logged in all the data, press **<CNTRL> <END>** as requested and the regression equation will be displayed, and, if you so indicate, saved.

If you wish to key in the body lengths from the keyboard, select "manual" callipers. You still require a calibration. In this case simply enter each actual stage micrometer increment.

4. SAMPLES

With the support files updated, the defaults set, and the callipers calibrated, you are now ready to count a sample. ZEBRA2 distinguishes a sample, i.e., the actual bottle of zooplankton, from a count of a sample. The first step is to describe the sample. ZEBRA2 provides two ways to do this from the Main Menu, the Sample Editor (Display/Edit) and the MOE disk.

Sample Display/Edit

Before logging in a sample, you must first select a data directory to put it in. The lower centre panel on the Main Menu indicates the directory you are in. To change to a data directory use the Misc. Directory option of the Main Menu.

Once you have selected a data directory, press **<Disp/Edit>** from the Sample panel on the main menu. You will be rewarded with a scrollable list displaying all the samples previously logged into this directory (e.g., Figure 12). If you wish to recount a sample already logged into the system, simply highlight it and press **<Enter>**. You can than move to the count editor. If you wish to delete a sample, press **<DEL>**. You can not delete a sample with active counts. If you wish to log a new sample into the system, press **<Ins>**. The Sample Data Entry Screen (Figure 13) shows such a screen for sample RCE.1, for example. Data describing the sample and the net or trap haul data are logged in separately.

Figure 12 - Illustration of Sample Records Screen

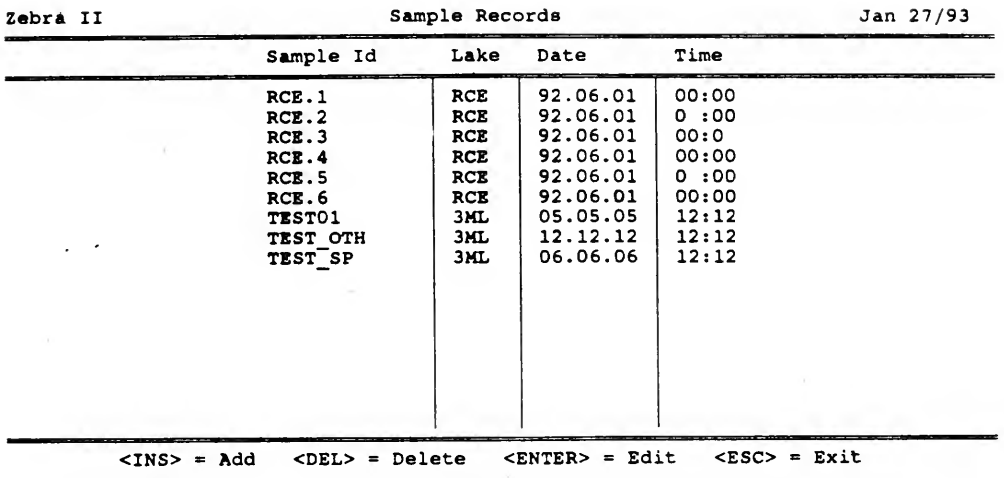
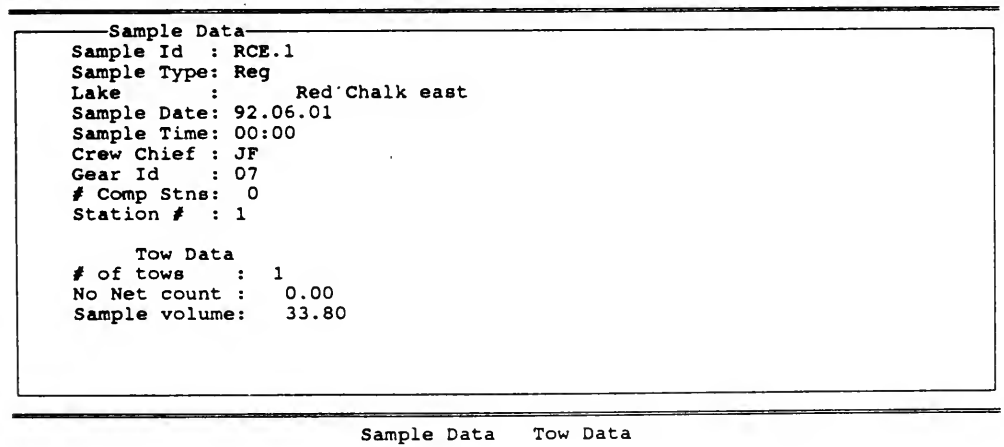


Figure 13 - Illustration of Sample Data Entry Screen



Sample Data

Key in all data in the Sample Records Screen as requested. You will be prompted for 9 parameters as follows:

SAMPLE ID is a 12 character field which can only be accessed when you are adding a new sample into the system; it cannot be edited later. The sample identifier must be unique. If it is not ZEBRA2 will beep at you and prompt for a new entry. Note that this variable refers to the sample, i.e., the bottle of animals, not to a count of the sample.

SAMPLE TYPE is a 5 place character variable that records the sample type. Feel free to create whatever sample type codes will be useful to you in the future, or to leave it blank. For example, a few of the current MOE DRC sample types include:

COMP1 - composite from several hauls at one station
COMP2 - composite from hauls at >1 station
DIS1 - sample from a discrete depth

There are many places within ZEBRA2 where the user is given the opportunity of logging in codes to record features of the samples, or counts of the samples. This is the first example of such a code. We strongly recommend using these features, but remind you that it is up to you to record the meanings of the codes. ZEBRA2 will not do it for you. Pawson and Yan (1993) provide a list and interpretation of all codes used by the MOE's DRC.

LAKE is a unique four character code assigned to each study lake logged into ZEBRA2. If you are logging in a new sample and you know the lake code you may simply type it in. Your entry will be checked against lake codes previously defined, verified, and the full name of the lake will appear on the screen. You may also

choose a previously defined code by pressing the **<F10> Lookup key** and scrolling through the pre-defined choices. Note that you cannot add a new lake code from the SAMPLE DATA screen. You can only do this from the Lakes editor accessed from the main menu.

SAMPLE DATE is the date that the sample was collected. The format shown on the screen (e.g., yy.mm.dd) is the format that you selected in the Defaults Screen.

SAMPLE TIME is the time that the sample was taken. Please enter in 24 hour format i.e., 14:00 for 2 pm.

CREW CHIEF is the initials of the chief of the field crew that collected the sample. We recommend you use the initials of the actual crew chief, not his or her supervisor back in the lab.

GEAR ID is a unique 2 digit code defined for each piece of zooplankton collection equipment. You may type it in, or select from the list displayed when you depress the **<F10>** key.

COMP STNS is a numeric variable recording the number of stations included in a spatial composite sample. This will be 1 in the case of a sample collected at a single station.

STATION # is the sampling station identifier code assigned by the sample collector. It should not be entered if a composite sample has been formed by pooling aliquots from more than one station.

When all the sample data have been entered, you will be prompted to **<R>e-enter**, **<A>ccept**, or **<C>ancel** the entry. If the entries were correct, type **<A>**. If you wish to edit the entries, press **<R>**, then **<Enter>** as many times as is required to highlight the faulty entry.

You will see this set of three options for verification of entries many times in ZEBRA2.

Tow Data

Once you have entered the sample data, you will be prompted to enter data on the "Tow". The program is looking for data on the numbers of hauls from particular depths, and, where appropriate, sample volumes or counts of plankton meters.

If you have entered a gear type of S/P, you will be prompted via pop-ups to enter the depth of the haul and the number of hauls from that depth. ZEBRA2 will then calculate the sample volume from the volume of the specified trap in the GEAR table and the number of hauls and display it for your verification.

If you have entered a gear type of OTH, for other miscellaneous net, ZEBRA2 will prompt you for the depth of hauls and the sample volume. If you know the filtration efficiency of the net, use it to calculate the actual lake water volume filtered.

If you have entered a gear type of C/B, ZEBRA2 assumes that you have sampled with a net made from a Clarke-Bumpus impellor modified as described in (Yan et al. 1992). This is the DRC's routine gear. It assumes you have a composite sample formed from combining the contents of various vertical hauls of various lengths. It will prompt you to enter the lengths and meter counts for each of these hauls. It will also ask you to enter the meter count for a "calibration" haul. Calibration hauls are assumed to be vertical hauls taken with the impeller itself (no net) through a distance equivalent to the longest sampling haul. ZEBRA2 then takes all of this haul information, and the mouth area of the identified gear

(from the Gear Table) and calculates the filtration efficiency and sample volume (see Appendix 3).

MOE Disk

The DRC often logs sample description information into their own master zooplankton database (Pawson and Yan 1993), then has the samples counted by non-resident technicians. The MOE disk feature was created so that descriptions of samples would not have to be re-keyed into a computer a second time.

The MOE disk feature will prompt for a source directory (usually a floppy disk) and a destination data directory, then log all of the sample and tow information from the source disk into data directory. There is an ancillary program supplied with ZEBRA2 called ZB_CKMOE. You can run this program to see how to set up the ASCII files to permit ZEBRA2 to access the data. The MOE disk feature populates the tables in a single data directory at a time. If you wish to have the data loaded into several data directories we recommend you supply the data in different sub-directories on the source diskette.

5. COUNTS

Now you are ready to actually count a sample. To begin, you must identify the count, using the Count Editor.

DISPLAY/EDIT accesses the Count Records screen (Figure 14). If you wish to delete or modify earlier counts press **<Del>** or **<Enter>**, respectively. To begin a new count, press **<Ins>**, and the Count Data pop-up screen (Figure 15) will appear. This screen prompts for information on nine count-related parameters.

Figure 14 - Illustration of Count Records Screen

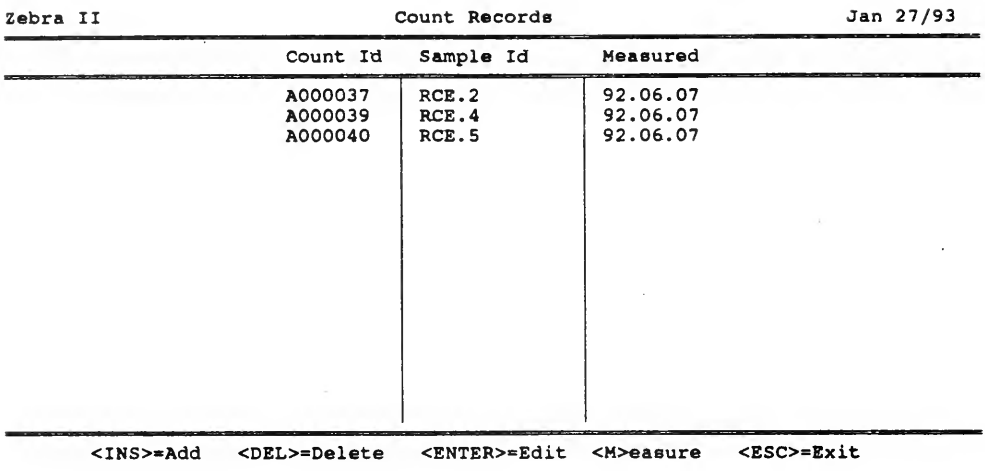
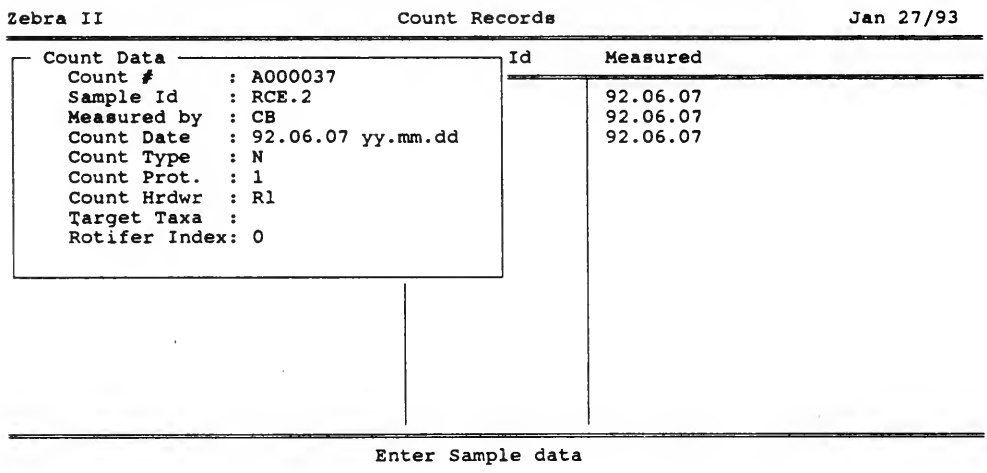


Figure 15 - Illustration of the Count Data Pop-up Screen



COUNT # is the unique identifier of the count. It is automatically assigned by the system to a new count of a sample, by which we mean a single complete session of identifying, enumerating, and measuring the targeted animals in a sample. The initial few characters of COUNT # will be the same for all counts in a user's system and are defined by the distributor of the software. The remaining numerics are assigned sequentially and can be used as an approximate indication of the total number of counts that have been made in this system.

SAMPLE ID records the unique identification code for a sample in the data sub-directory. If you have accessed this screen from the Count editor, and are starting a new count, you can hit **<F10>** to display a list of the available samples. You can not count a sample that has not been previously described in the sample editor.

MEASURED BY is the code of the name of the sample enumerator, i.e., you.

COUNT DATE is the date you started this count of the sample (entered in your chosen date format).

COUNT TYPE records the "type" of count. The default type can be altered from the Defaults option in the Main Menu. As an example of count type codes, those used by the MOE's DRC include:

R - a routine count of a sample
QAQC1 - replicate count with the same count protocol
QAQC2 - repeated count with a different protocol
SS - a special count

COUNT PROT records the counting protocol employed during the count. For example, some of the count protocol codes used by the MOE's DRC include:

R1 - Entire sample counted

R2 - total count fixed at 350 animals

R3 - total count fixed at 250 animals, <50 per taxon, <30 per nauplii

COUNT HRDWR is a code you create to record the measurement and magnification hardware used during the count. An example code could be N_S1 for Nikon microscope, scissor callipers number 1.

TARGET TAXA is a code that records the group of taxa targeted in the count. For example, the codes used by the MOE's DRC include:

CRUST - count of crustacean zooplankton

CHAOB - count of larval *Chaoborus*

ROT - count of rotifers

C_R - count of Crustacea and rotifers

CCR - count of Crustacea, *Chaoborus* and rotifers

ROTIFER INDEX is a ordinal ranking of the abundance of all rotifers in the count, during a count in which rotifers are not, themselves, being counted. Its intent is to provide a rough, but permanent index of rotifer abundance in the sample. As used by the MOE's DRC, the ROTIFER INDEX takes on only the integer values 1 through 5.

When you have verified the count parameters, by pressing **<A>ccept**, a comment editor screen will appear. If you wish to log in any comment about the count, this is your first opportunity. You can also do this at any time during the count. Having done this you are now ready to begin the actual count. Press <M> to get to the Measurement Screen.

Measurement

Most of the counting action takes place within this screen. Figure 16 shows the appearance of this screen after a single 0.825 mm long *Daphnia dubia* has been counted. The top line of the screen provides the identity codes for the COUNT, the SAMPLE, the LAKE and the TOTAL COUNT. The last parameter is the only one on this line whose value changes during a count. It records the current total number of animals that have been identified and measured.

Figure 16 - Illustration of Measurement Screen During a Count

```
Zebra II                          Measurements                          Jan 27/93
Count  Id:          Sample:                  Lake: RCE      Total Count:     1
```

Zoom	Code	Species	Length	Weight	
2.0					

```
###    Spp    Len(mm)                          Summary
  1    121    0.8250000   121 Da. dubia    1

[A]ttrib  [C]omment [D]el  [F]ract  [P]ower  [N]ew Sp. [R]otifer Rank  [Z]ap
 Press <SPACE> for new measurement, or <+> to add to a previous measurement
```

Below this is a box showing the current microscope Magnification Factor (the ZOOM Ring setting), the Species Identity CODE, and abbreviated identity (SPECIES) of the animal being measured, and the LENGTH and calculated WEIGHT of that animal.

The Magnification Factor (ZOOM) requires some explanation. The user will frequently alter the magnification setting of the microscope so that the image of the animal or the body part being measured is a satisfactory size. ZOOM records this setting.

The system was developed on a Nikon SM-Z10 dissecting scope with Zoom Ring settings of 1 to 4. These settings indicate relative not absolute magnification, i.e., the image on setting 4 is twice as large as on setting 2. The Magnification Factor parameter records the actual Zoom Ring setting, if an SM-Z10 is being used to count the sample. It should be used to record the analogue of SM-Z10 settings if some other microscope is used to count the sample. For example, if the zoom ring on your microscope records actual magnification values of say 10 to 50 times, and you have calibrated the system at 25x, then set this parameter to 2 in ZEBRA2 if you are counting the sample at 50 fold magnification.

On the left hand side of the display, below the box giving the Magnification Factor, is a scrollable list of the individual animal's number (###), Species identity codes (SPP) and associated lengths (LEN (MM)) of all animals counted and measured in the session. The number of the animal is assigned to that animal permanently. If a number is missing, a measurement has been deleted by the user for some reason.

To the right is the current summary of the counting session. It records the identity and numbers of each species counted and measured during the count. Sub-sample volumes examined can differ among species in a count; hence, the fraction of the total sample examined for each species must be recorded. Those for which a FRACTION ANALYZED has been recorded will have an asterisk between the 3-digit Species Id code (121 in Figure 16) and the abbreviated name of the species. To complete a count, a Fraction Analyzed must be entered for each species.

At the bottom of the screen is an extensive menu which allows you to efficiently accomplish a number of tasks. Note that if you are using Vernier callipers, no menu action is required to measure an animal - simply press the button on the callipers. If you are using the Sprules

Scissors callipers, press the **<Space Bar>** when you wish to record a measurement.. For those of you familiar with the previous version of Zebra, this version is in permanent "Repeat Mode" i.e., any measurements made are assumed to belong to the species currently indicated in the box near the top left of the screen. To change species, press **<N>**ew as described below. You can use the UP and DOWN cursor keys to highlight individuals in stack for deletion, addition of attribute information, etc. If you can not remember the identity code of the species, use **<F10>**. If you remember the species is in the 200 series, enter 2 and the look-up will advance to the 200 series. Remember that you can page through the look-up, or use **<Ctrl>** **<PgDn>** or **<Pgup>** to move to the end or beginning of the look-up.

The actions of the various menu selections are as follows:

<A>ttrib allows you to record attributes of the currently highlighted individual other than its body length. By attributes we mean any numeric feature, such a egg count, measured value, such as mucron length, value selected from a table giving all possible values, such as male gender, or typed text. You will be asked to select an attribute from all possible attributes previously specified in the Attributes option under Tables in the Main Menu. Once you have identified the attribute you will be asked to key in the value for a Numeric or Character attribute, measure a Measured attribute, or highlight the correct entry from a pop-up of Tabled attributes. Remember to adjust ZOOM from outside the attribute routine (using **P**) if you have adjusted the magnification to enlarge the size of a measured attribute on your monitor.

<C>omment accesses the comments screen you first saw in the Count editor. This is the place to record any comments concerning this count of the sample. Were you unsure of any of the taxonomy? Were any of the animals badly preserved? Was there anything unusual in the count, e.g., parasitized animals, lots of loose eggs, copepods with lots of spermatophores? Do not use this feature to record a comment

you wish to associate with an individual animal. Use the Attribute feature for such a purpose. This count comment is saved in the Count file and will appear on the Bench sheet.

There is really no practical limit to the length of comment you are allowed to record. A scrollable window will pop up which can hold up to 64,000 characters. In order to save any changes you make, press **<Ctrl><W>** (remember W for **Write**). Pressing **<ESC>** will abort any changes you have made.

<D>elete deletes any highlighted entry from the stack of measurements. You will be asked to confirm your intent. Note that the species and total counts will reflect the deletion, and a break in the sequence of numeric animal identifier codes (**###**) will result.

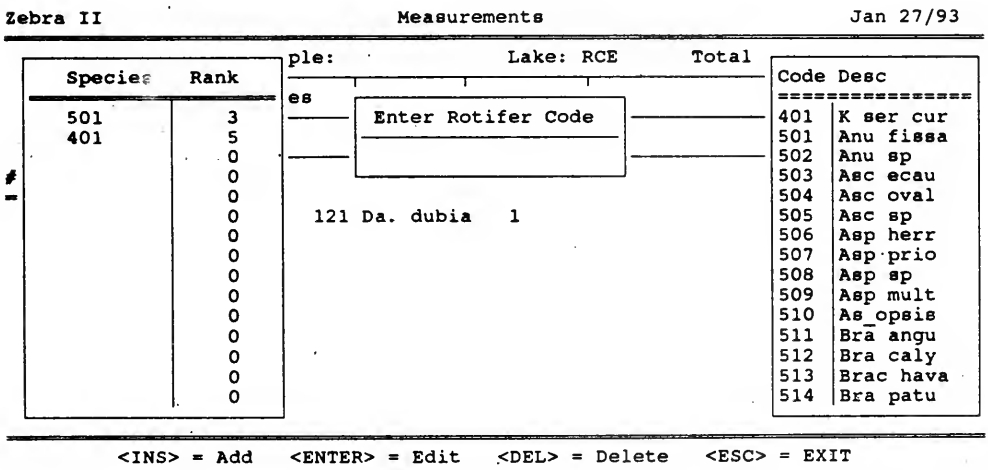
<F>rac allows entry of the FRACTION ANALYZED for any individual species or, collectively, for all remaining species. This number, which must be between 0 and 1, records the proportion of the entire sample that has been examined for the indicated species. A count is not considered complete unless a Fraction Analyzed has been entered for all taxa encountered in the session. If the fraction analyzed is the same for all remaining taxa simply enter **<***>** into the pop-up requesting species identity.

<P>ower allows you to change the current Magnification Factor (ZOOM). In case you are wondering, the reason we didn't use <M>agnif is because the **<M>** and <N> keys are side by side on the keyboard and you have to use **<N>** fairly often.

<N>ew Sp. allows you to change species. Remember you can look up species using **<F10>**.

<R>otifer Rank allows you to enter an estimated rank for an individual rotifer species via a pop-up window (see Figure 17). We put this feature in to record impressions about rotifer relative abundances, when Crustacea were the focus of the count. Naturally, you would not use this feature if you were targetting rotifers in the count. You will be asked to select a rotifer species and record its estimated rank abundance. Naturally, you can access the Rotifer Species table by pressing **<F10>** in this window. This produces the rotifer species look-up panel up the right side of Figure 17.

Figure 17 - Illustration of Rotifer Ranking and Rotifer Species Look-up PopUp Screens



<Z>ap allows you to delete all the measurements for an entire species. You will be asked for confirmation to avoid accidentally deleting a lot of work!

<+> allows you to add an additional length increment to an individual, for example, to measure a bent copepod in sections.

ZEBRA2 records all activities on disk immediately, so no explicit SAVE function is required. When you press <ESC> to exit this screen, rest assured that your data are already safely tucked away.

Bench sheet

This routine permits the printing of a summary of a specified count. The default is the current count. The header of the bench sheet provides the sample and count information and comments. Its body provides the species identifier code and abbreviated name, sub-sample volume examined (fraction analyzed), numbers counted, density (i.e., abundance), mean length, mean weight and biomass of each taxon observed in the count. The numbers of all species and individuals processed, and total density and biomass are also displayed. A bench sheet for a small demonstration count is provided in Figure 18. See Appendix 3 for formulae used to calculate these parameters.

Figure 18 - Sample Bench Sheet

Zooplankton Bench Sheet for Sample #: ZB920622 Printed: 93.01.27 @14:57

Lake: 3ML (Three Mile)
Sample Date: 92.06.22 @10:10
Crew Chief: gga Sta #:
Samp. Vol.(L): 76.97
Gear Code: 02 (C/B Metered Net)
Mesh (um): 76
Tow (m): 2.0 3.0 4.0 5.0
Counts : 4.0 5.9 8.0 9.9
Ratio :1.98 1.97 2.00 1.98

Count #: A000024
Count Date: 92.06.22
Counter: NDY
Type: N Protocol:
Count Hardware: RG
Rotifer Index: 1 Taxa :

Efficiency (%): 45.06 Calib. Count: 22.00

Comments:
test

Zooplankton Code	Name	Fract. Anal.	#/ Fract.	Density (#/m^3)	Mean Length (mm)	Mean Weight (ug)	Biomass (mg/m^3)
101	Ac curv	0.100000	1	129.9	0.317	1.446	0.188
110	Bos long	0.100000	6	779.5	0.598	5.969	4.653
111	Ce. lacus	0.100000	1	129.9	0.695	1.782	0.231
121	Da. dubia	0.100000	4	519.7	0.437	0.497	0.258
122	Da g men	0.100000	1	129.9	0.988	4.834	0.628
135	H gibber	0.100000	5	649.6	0.739	5.153	3.348
201	Cal copep	0.100000	1	129.9	0.539	1.205	0.157
204	Lepto minu	0.100000	7	909.4	0.911	6.306	5.735
304	Cyc vern	0.100000	1	129.9	0.746	2.679	0.348
305	Erg sp	0.025000	1	519.7	0.605	1.599	0.831
TOTALS:	10		28	4027.5			16.377

Import

This routine permits the loading of files generated by an earlier version of ZEBRA into the files of this version of the program. Users who are new to ZEBRA2 will never need this routine. If you are importing the files from ZEBRA1, make sure to set up the destination data directories carefully. At the moment you cannot move data between data directories in ZEBRA2.

Export

This is the routine that permits the export of data from the system to a floppy disk for archival purposes or to generate data sub-sets to be imported into some other database. You can tag data to export by individual count identifier, by count date, or by lake. The marked data can be optionally purged from your system once it is exported. You will be asked to identify a path for the export.

The product of Export is a set of Dbase data files (see Appendix 1) that contain the count information you have specified. These files can then be manipulated in any program that can read Dbase files, for example, the spreadsheets QUATTRO and EXCEL.

Resume

This routine allows the user to return to the measurement screen of the last counting session with a single key stroke. This feature greatly facilitates the interruption and restarting of counts.

Final Comment

Finally, if you are having trouble, remember the Help Screens **<F1>** and the Look-ups for tabled entries **<F10>**. If you are still having trouble, feel free to call.

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Appendix 1 ZEBRA2 File Structures

Appendix 1 documents the structure of all of the ZEBRA2 Dbase files. The System files (A) contain no sample or count data and need never be examined by most users. These files reside in the ZEBRA2 directory. The Base files (B) retain information on the attributes, gear, species and lakes accessed through the Tables option in the main menu. Information on the calipers, and various default settings are also stored in these Base files. The Data Tables (C) are located in the data sub-directories. All of the information on the samples and their counts are stored in these tables.

For each file we provide the Field Number (Fld), Field Name (FldNam), Field Type (Typ) (C-Character, N-Numeric, M-Memo, D-Date), Field Width including decimals (Wid), number of decimal places (Dec) and a brief description.

A. System Files

ZB_DBF.dbf - file dictionary

Fld	FldNam	Typ	Wid	Dec	
001	F_NAME	C	8	0	File Name
002	F_ALIAS	C	8	0	Program Alias
003	F_NTX1	C	8	0	Primary Index
004	F_NTX2	C	8	0	Secondary Index
005	F_NTX3	C	8	0	Tertiary Index

ZB_HLP.dbf - help dictionary

Fld	FldNam	Typ	Wid	Dec	
001	VAR	C	10	0	Variable Name
002	DESC	C	25	0	Description of Variable
003	VARLEN	N	2	0	Length of Variable
004	VARFORM	C	25	0	Format of Variable
005	MAN_PG	N	3	0	Page in Manual
006	COMMENT	M	10	0	Help Text

ZB_NTX.dbf - index dictionary

Fld	FldNam	Typ	Wid	Dec	
001	DBF_NAME	C	12	0	Database File Name
002	NTX_NAME	C	8	0	Index File Name
003	NTX_EXPR	C	40	0	Index Expression

ZB_LU.dbf - look up dictionary

Fld	FldNam	Typ	Wid	Dec	
001	LUVAR	C	10	0	Variable Name
002	LUPROC	C	10	0	Procedure or Function Name
003	LUALIAS	C	8	0	Alias of Lookup File
004	LUKEY	C	10	0	Key Field for Lookup File
005	LUDESC	C	60	0	Fields to Display
006	LURTN	C	10	0	Field to Return
007	LUNTX	N	2	0	Index Order for Lookup File
008	LUFLTR	C	40	0	Filter Expression for Records

ZB_MNU.dbf - program menu selections

Fld	FldNam	Typ	Wid	Dec	
001	MNU	N	1	0	Main Menu Number
002	ITM_NO	N	2	0	Item # in Menu
003	MNU_TXT	C	12	0	Text to Display
004	MNU_CHK	C	8	0	Check Function
005	MNU_PRG	C	10	0	Program Function

ZB_PRN.dbf - printer configuration file

Fld	FldNam	Typ	Wid	Dec	
001	PRINTER	C	20	0	Printer Name
002	BLDON	C	10	0	Bold On
003	BLDOFF	C	10	0	Bold Off
004	COL80	C	10	0	10 cpi
005	COL96	C	10	0	12 cpi
006	COL132	C	10	0	16.67 cpi (compressed print)
007	ULON	C	10	0	Underline On
008	ULOFF	C	10	0	Underline Off
009	ITALON	C	10	0	Italics On
010	ITALOFF	C	10	0	Italics Off
011	WIDON	C	10	0	Double Width Letters On

012	WIDOFF	C	10	0	Double Width Letters Off
013	LPI6	C	10	0	6 Lines per Inch
014	LPI8	C	10	0	8 Lines per Inch
015	PGLENSET	C	10	0	Page Length Setting
016	RESET	C	10	0	Printer Reset
017	PGLEN	N	3	0	Normal Page Length
018	MAXROW	N	3	0	Maximum Row to Print
019	OFFSET	N	2	0	Top Margin
020	DRAFT	C	10	0	Printer Code for Draft Quality
021	LQC	C	10	0	Printer Code for Letter Quality

ZB_SCR.dbf - screens file

Fld	**FldNam**	**Typ**	**Wid**	**Dec**	
001	SCRNAME	C	10	0	Screen Name
002	S_ROW	N	2	0	Screen Row
003	S_COL	N	2	0	Screen Column
004	S_LINE	C	80	0	Text to Display

B. Zebra Base Files

ZB_ATTR.dbf - attributes file

Fld	**FldNam**	**Typ**	**Wid**	**Dec**	
001	CODE	C	4	0	Attribute Code (internally generated)
002	DESC	C	20	0	Attribute Description
003	TYPE	C	1	0	C = character N = numeric M = measure T = table entry

ZB_ATTBL.dbf - values of tabular attributes

Fld	**FldNam**	**Typ**	**Wid**	**Dec**	
001	CODE	C	4	0	Attribute Key
002	ENTRY	C	20	0	Attribute Value

ZB_CALIB.dbf - calibration records (one for each set of callipers)

Fld	FldNam	Typ	Wid	Dec	
001	CAL_ID	C	5	0	Calliper Identification Code
002	CAL_DATE	D	8	0	Calibration Date
003	CAL_TIME	C	5	0	Calibration Time
004	OPERATOR	C	5	0	Operator
005	MAGNIF	N	5	3	Microscope Zoom Ring Setting
006	SLOPE	N	9	4	Slope of Calibration Curve
007	INTERCEPT	N	9	4	Intercept of Calibration Curve
008	R2	N	7	5	Coefficient of Determination

ZB_CALIP.dbf - master calliper file

Fld	FldNam	Typ	Wid	Dec	
001	CAL_ID	C	5	0	Calliper Identification Code
002	CAL_TYP	C	2	0	Calliper Type (Fowler, manual, scissors)
003	CAL_DESC	C	20	0	Calliper Description
004	CAL_PORT	C	10	0	Port Number
005	CAL_CALC	C	30	0	Code Block for Conversion of Reading to Measurement

ZB_DFLT.dbf - system defaults

Fld	FldNam	Typ	Wid	Dec	
001	INSTDATE	D	8	0	Installation Date
002	MODDATE	D	8	0	Date Last Modified
003	DATEFORMAT	C	8	0	Date Format (yy/mm/dd etc.)
004	SYSPWORD	N	8	0	System Password
005	PR_LOC1	C	20	0	Local Printer # 1
006	PR_LOC2	C	20	0	Local Printer #2
007	PR_NW1	C	20	0	Network Printer #1
008	PR_NW2	C	20	0	Network Printer #2
009	SYSLOCK	N	8	0	Encrypted Serial #
010	DEF_FLOPPY	C	2	0	Default Floppy Drive Code
011	CODEPATH	C	25	0	Path to System Files
012	DATADIR	C	25	0	Path to Directory for LASTSMPL
013	BELLTONE	N	4	0	Speaker Frequency (MHz) for Beeps
014	BELLTIME	N	2	0	Length of Beeps (seconds)
015	CAL_ID	C	5	0	Default Calliper ID Code

016	MEAS_BY	C	5	0	Default Initials of Sample Enumerator
017	MAGNIF	N	5	3	Default Zoom Ring Setting
018	LASTDIR	C	40	0	Directory for Last Sample Counted
019	LASTCNT	C	7	0	Last Count ID Code
020	NXTCNT	C	7	0	Next Count ID to be Assigned
021	CNT_TYPE	C	5	0	Default Count Type
022	CNT_PRTCL	C	5	0	Default Count Protocol
023	CNT_HRDWR	C	5	0	Default Count Hardware

ZB_GEAR.dbf - Gear description file

Fld	FldNam	Typ	Wid	Dec	
001	GEAR_ID	C	2	0	Gear Identification Code
002	GEAR_TYP	C	5	0	Gear Type (CB/SP/OTH)
003	GEAR_DESC	C	25	0	Gear Description
004	MESH	N	4	0	Mesh Aperture Size (um)
005	DIAM	N	6	4	Diameter (metres)
006	AREA	N	6	4	Area (sq. metres)
007	VOL	N	6	4	Volume (cubic metres)

ZB_LAKES.dbf - Lake definition file

Fld	FldNam	Typ	Wid	Dec	
001	LAKE_COD	C	4	0	Lake Code (used for operator entry)
002	LAKE_NAM	C	30	0	Lake Name

ZB_SPEC.dbf - Species definition file

Fld	FldNam	Typ	Wid	Dec	
001	SP_CODE	C	3	0	Species Code
002	SP_ABBREV	C	10	0	Species Abbreviation
003	SP_NAME	C	35	0	Species Latin Binomial
004	MIN_LEN	N	6	3	Minimum Length
005	MAX_LEN	N	6	3	Maximum Length
006	WA_MULT	N	7	4	A in wt = $A.len^B$
007	WB_EXPON	N	7	4	B in wt = $A.len^B$

C. Data Directory Tables

ZB_CATTR.dbf - Individual organism attribute file (one record per attribute)

Fld	FldNam	Typ	Wid	Dec	
001	CNT_ID	C	7	0	Count identification Code
002	INDIV_ID	N	4	0	Individual Organism #
003	ATTRIB	C	20	0	Attribute value for organism
004	CODE	C	4	0	Code of attribute

ZB_CDTL.dbf - Count detail file (one record/species in the count)

Fld	FldNam	Typ	Wid	Dec	
001	CNT_ID	C	7	0	Count Identification Code
002	SP_CODE	C	3	0	Species Code
003	FRAC_ANAL	N	8	6	Fraction of Sample Analyzed (%)
004	ORG_CNT	N	4	0	# of Organisms Counted
005	SUM_LEN	N	11	7	Sum of Lengths of the species
006	SUM_WT	N	11	4	Sum of Weights of the species
007	MIN_LEN	N	7	3	Minimum Length Allowed (mm)
008	MAX_LEN	N	7	3	Maximum Length Allowed (mm)
009	WA_MULT	N	6	3	A in wt = $A.len^B$
010	WB_EXPON	N	6	4	B in wt = $A.len^B$

ZB_CHDR.dbf - Count header file (one record per count)

Fld	FldNam	Typ	Wid	Dec	
001	SMPL_ID	C	12	0	Sample Identification Code
002	TXT_FILE	C	12	0	Name of Zebra Text File
003	MEAS_BY	C	5	0	Initials of Sample Enumerator
004	ROT_INDX	N	1	0	Ordinal Rotifer Abundance
005	COMMENT	M	10	0	Comment Field
006	CNT_DAT	D	8	0	Date Counted
007	CNT_ID	C	7	0	Count Identification Code
008	SMPL_TAG	C	1	0	Tag Field
009	CNT_TYPE	C	5	0	Count Type
010	CNT_PRTCL	C	5	0	Count Protocol
011	CNT_HRDWR	C	5	0	Count Hardware
012	CNT_PHYLM	C	5	0	Taxa Targetted in Count

ZB_CMSR.dbf - Animal measurements file (one record per measurement)

Fld	FldNam	Typ	Wid	Dec	
001	CNT_ID	C	7	0	Count Identification Code
002	SP_CODE	C	3	0	Species Code
003	MEAS	N	9	7	Length of Organism
004	INDIV_ID	N	4	0	Individual Organism Number

ZB_ROTIF.dbf - Dominant rotifer file (one record per taxon)

Fld	FldNam	Typ	Wid	Dec	
001	CNT_ID	C	7	0	Count Identification Code
002	SPEC_ID	C	3	0	Species Code
003	RANK	N	2	0	Rank

ZB_SMPL.dbf - Sample header file (one record per sample)

Fld	FldNam	Typ	Wid	Dec	
001	SMPL_ID	C	12	0	Sample Identification Code
002	TXT_FILE	C	12	0	Name of Zebra File Imported
003	LAKE_COD	C	4	0	Lake Identification Code
004	SMPL_DAT	D	8	0	Date of Sampling
005	SMPL_TIM	C	5	0	Time of Sampling
006	GEAR_ID	C	2	0	Gear ID Number
007	NUM_TOWS	N	2	0	Number of Tows with Gear
008	WO_COUNT	N	6	2	Impeller Count of Calibration Haul
009	SMPL_VOL	N	7	2	Sample Volume (litres)
010	CR_CHIEF	C	5	0	Crew Chief
011	EFFIC	N	6	2	Efficiency (%) of Filtration of Haul
012	SMPL_TYPE	C	5	0	Sample Type
013	COMP_STN	N	2	0	Number of Stations in Composite
014	DEPTH	N	6	2	Depth (metres) of Haul
015	STN_NUM	C	3	0	Station Number

ZB_STOW.dbf - Tow records file (one record/tow)

Fld	FldNam	Typ	Wid	Dec	
001	SMPL_ID	C	12	0	Sample Identification Code
002	TOW_LEN	N	6	2	Length of Tow (metres)
003	TOW_METRE	N	6	2	Impeller Count for Tow

Appendix 2 Useful Editing Keys in CA-Clipper Programs.

Keyboard Usage:

Presumably there are fewer and fewer "computer novices" around these days, however, whether you put yourself in this category or not, you may find the following information useful. It includes some general information on computer keyboards, some peculiarities of keyboard usage in CA-Clipper programs, and points out some of our own idiosyncrasies.

In general, the program accepts upper or lower case as equivalent characters. The place where the characters that you type will appear is indicated by a cursor. This is usually a blinking underline, but may be a blinking block depending on the machine and whether it is **Insert** or **Typeover** mode.

Keyboard Characters:

<Enter> or <Return> is called the carriage return key and behaves much like the same key on a typewriter in that it usually advances you to the next line on the screen, or in the case of a data entry screen, to the next item. Note that during data entry, pressing the <Enter> key when the cursor is anywhere in the field will enter the entire field.

Your computer keyboard has several keys not found on a normal typewriter. Two of these, CTRL and ALT are like extended SHIFT keys: they are usually used in conjunction with another key. This is represented in the manual by: e.g., <CTRL><G> (no space between the keys) which indicates that you should hold down the CTRL key while pressing the G key.

The cursor keys include the arrow keys, PgUp, PgDn, HOME and END. These are used for moving around on the screen. You may notice that there are two keys with an arrow pointing to the left. The one towards the upper right hand corner above the ENTER key deletes the character to the left of the cursor, while the one grouped with the other arrow keys simply moves the cursor to the left.

Function keys are labelled F1 to F10 (up to F12 on some keyboards). Their purpose is defined by the program you are running, although the use of F1 as the HELP key is a common standard and ZEBRA2 follows this convention. The F10 key in ZEBRA2 is defined as the LOOK UP key and allows you to "pop up" a window containing potential entries for the parameters requested by the program.

ESC is called the ESCAPE key and in ZEBRA2 is allows you to abort whatever operation you are performing at the time. It is also used to exit from menus.

INS is the INSERT key and is toggled on or off each time it is pressed. While it is ON (as indicated by a square block cursor) anything you type will be inserted into the text on the screen starting at the current cursor position. While it is OFF (indicated by an underline cursor) characters that you type will overwrite those on the screen at the current cursor position.

Menu Selection:

Many ZEBRA2 functions are reached from "Light Bar Menus" in which several options are presented, one of which is highlighted. The cursor keys can be used to move the highlight to the desired function and <ENTER> pressed to initiate the action. In most cases, you can also make your choice by pressing the key which matches the first character of the word. For example, the following menu always appears after filling in the fields of a screen display:

Re Enter Accept Cancel

the initially highlighted entry will likely be **Re Enter**.

Press <R> to correct the data before saving.

Press <A> to save the data as entered. If you notice an error after saving, you can correct it by using the EDIT function available on all data entry screens. For those of you who have had experience with the old version of ZEBRA, it may be useful to note that your data has now been written to disk - you will not lose it if the power should fail!

Press <C> to cancel your changes.

One other type of menu used at the bottom of a screen is similar except that highlighting is not used. Selections here are made only by pressing the letter on the keyboard which matches the first character of the desired selection. In order to make this clearer these characters are presented in the < > brackets.

e.g., <A>dd <D>elete <E>dit <S>earch <Q>uit

The ESC key will allow you to exit from any menu selection.

Editing Shortcuts:

Those of you who have used WordStar or Sidekick will be accustomed to using Control Key combinations for editing. Several of these combinations may be used in ZEBRA 2:

<CTRL>	in conjunction with the <E>, <D>, <X> and <S> keys will move you up, right, down or left, respectively.
<CTRL><G>	will delete the character at the current cursor position.
<CTRL><H>	will delete the character to the left of the cursor.
<CTRL><T>	will delete the word to the right of the cursor.
<CTRL><Y>	will delete from the cursor to the end of the current field.
<CTRL><W>	will accept all of the data within the scope of the current "read". This may vary from only the current field to all of

the data on the current screen. (This is more closely defined in the descriptions of the individual screens.)

As confidence and typing speed increase, you may discover a feature known as the "TYPE AHEAD BUFFER". Often, while the computer is busy processing the last characters that you have typed in, you may continue to enter commands or data until the computer beeps. They will not appear on the screen but the computer will remember what you have typed and process them one by one until it runs out of characters. The capacity of the type ahead buffer is usually 9-15 characters. You will learn from experience that the type ahead buffer is not always available to you. For instance, it would be awkward if you were to miss significant error messages because the characters in the buffer removed the information from the screen before you had a chance to read it.

Error Handling

Program Errors:

Standard computer screens are 80 characters wide and 25 lines deep with the lines numbered 0 (at the top) to 24 (at the bottom). During program execution lines 0 and 24 are reserved for system use. Occasionally, messages will appear on these lines as a result of incorrect input, disk failure or to advise you of system activity. If a message appears on line 0 in the format:

Proc:FILEMAIN line: 2312 File not Found

you should do two things immediately:

1) make sure that your printer is on line; and

2) hold down the <SHIFT> key and press the <PrtSc> key. This will dump the contents of the screen to the printer for future reference.

This should be a very rare occurrence: it means that either we have a "bug" in our program or your machine has generated some kind of internal error. In any case, it would be a good idea to inform your local support person of the problem before continuing.

Data File Errors:

Although you enter data into files in random order, they almost always appear on the screen in an organized fashion, such as alphanumeric order. This is accomplished by the use of index files which the program maintains automatically. If you turn off your machine in the middle of a program or experience a power disruption, damage is most likely to happen to these index files. The most obvious symptoms of this are:

1) not being able to find data you know should be in the system, or
2) data appearing on your screen does not appear to be in the correct order.

In either case, the problem can generally be rectified by running the re-indexing routine which can be accessed from the Main Menu. It is important to note that separate index files are kept for each of your directories. You should run Index Maintenance while the current directory is your ZEBRA2 directory to re-index the system files and then rerun Index Maintenance in the data directory that contains the problem files.

Appendix 3 Selected Formulae Employed Within ZEBRA2.

Following are a number of formulae used within ZEBRA2. Parameters are listed as the file name to the left of the decimal with the Fieldname (variable) to the right. All variables are described in Appendix 1.

For the Bench Sheet

Sample volume (Vol) if Gear ID = "C/B" (See Locke & Scott (1986))

$$\text{Vol} = (\text{ZB\_GEAR.AREA}) \times (\Sigma\ \text{ZB\_STOW.TOW\_METRE}) \times (\text{MAX(ZB\_STOW.TOW\_LEN)}) \div (\text{ZB\_SMPL.WO\_COUNT}).$$

Sample volume if Gear ID = "S/P"

$$\text{Vol} = \text{ZB\_GEAR.VOL} \times \text{ZB\_SMPL.NUMTOWS}$$

$$\text{Density} = \frac{n}{\text{FA} \times \text{VOL}}$$

where n = ZB_CDTL.ORG_CNT
FA = ZB_CDTL.FRAC_ANAL
VOL = ZB_SMPL.SMPL_VOL

$$\text{Mean Length} = \frac{\Sigma L}{n}$$

where ΣL = ZB_CDTL.SUM_LEN
n = ZB_CDTL.ORG_CNT

Mean Weight = $\frac{\Sigma W}{n}$

where ΣW = ZB_CDTL.SUM_WT

n = ZB_CDTL.ORG_CNT

Biomass = $\sum_{i}^{m} \left(\frac{\sum_{c}^{n} (w_i)}{FA_i \cdot VOL} \right)$

where m = Count (SP_CODE) in CNT_ID

n = ZB_CDTL.ORG_CNT

FA = ZB_CDTL.FRAC_ANAL

VOL = ZB_SMPL.SMPL_VOL

For the Measurement Screen

Weight = aL^b

where a = ZB_SPEC.WA_MULT

b = ZB_SPEC.WB_EXPON

L = ZB_MSR.MEAS